REMARKS

Proteins are needed for a variety of uses, especially for commercial food and chemical production and for medically therapeutic uses. There is a continuing need for improved cellular expression systems that produce higher yields of these target heterologous proteins and that can be subjected to relatively easy production and purification techniques. The present invention provides a protein, a polynucleotide sequence encoding the protein, and a method of using the protein to increase intracellular production or secretion of a target heterologous protein in a cellular expression system. In particular, a novel protein, vesicular fusion factor 2 (Vff2p) and variants thereof have been discovered and isolated. In addition, a method for using these proteins to increase intracellular protein or secreted protein in a cellular expression system has been discovered.

Applicant has carefully reviewed and considered the Office Action mailed on December 15, 2000, and the references cited therewith.

Claims 1, 2, 13, 24, 28, and 35 are canceled; claims 3-6, 8, 12, 14-17, 19, 23, 25, 26, 29-31, 33, 34, 36, and 37 are amended; and claims 43-46 are added. As a result, claims 3-12, 14-23, 25-27, 29-34, and 36-46 are now pending in this application. No new subject matter has been added. The cancellations and amendments have been made to expedite prosecution of the present application and not for reasons of patentability. Applicants wish to make clear that the subject matter no longer pursued in the present application may be pursued in continuing applications. The amendments are made to clarify the claims, and not for reasons relating to patentability. Therefore, the amendments are not intended to limit the scope of equivalents to which any claim element may be entitled. The amendments to the claims are fully supported by the specification as originally filed.

Claims 5, 6, 8, 16, 17, 19, 29, and 30 have been amended to change dependencies due to the cancellation of claims.

As claims 1 and 2 have been cancelled, previously-dependent claim 3 has been amended to recite the features of claims 1 and 2; claim 14 has been amended to recite the features of cancelled claim 13; claim 36 has been amended to recite the features of cancelled claim 35. As claim 24 was cancelled, claim 25 has been amended to be an independent claim. Claims 14, 25 and 36 have been amended to recite the features of originally-filed claim 2.

Claims 3, 14, 25, and 35 have also been amended to recite that the Vff2p can be SEQ ID NO:2 or a variant thereof. The term "variant" of Vff2p is a term readily recognized by those of

skill in the art and is, for example, defined in the specification as a polypeptide that is not completely identical to native Vff2p. *See*, for example, specification at page 9, line 7 through page 10, line 28.

Claims 4, 15, and 26 have been amended to correct dependency and to recite that the polynucleotide can be SEQ ID NO:1 or a variant thereof. It should be noted that a "variant" of the *VFF2* nucleotide sequence is, for example, defined in the specification as a functionally equivalent insertion, deletion and substitution sequence of *VFF2* having an insertion, deletion or substitution sequence. *See*, for example, specification at page 4, line 28 through page 5, line 1; and page 8, line 17 through page 9, line 6. In particular, an insertion or deletion can be made to non-essential regions of the *VFF2*. *Id. See also*, for example, page 11, lines 1-11 of the specification, wherein further variants of *VFF2* are identified by Applicants.

A polynucleotide sequence of the present invention is a sequence encoding a functional Vff2 protein. It is well known that one or more nucleotide substitutions can be made to a polynucleotide sequence without affecting production of a functional protein. It is also known that additional nucleotides can be added either 5′ or 3′, or both, to a polynucleotide sequence without affecting expression of a functional protein from that polynucleotide. Therefore, the present invention includes modifications of a polynucleotide sequence of SEQ ID NO:1, wherein that sequence has been modified by any of the changes previously discussed, has been modified internally, or wherein that sequence or modification thereof has been linked to added 5′ or 3′ nucleotides without changing the functionality of the protein expressed therefrom. *See* specification at page 8, line 17 to page 9, line 6.

Claims 12, 23, 32 and 34 have been amended to clarify grammar.

Claims 31 and 32 have been amended to recite a method for increasing cell growth of a host cell. Support for this amendment is found in the specification, for example, at page 25, lines 1-9. Support for the recitation in claims 31 and 33 that the Vff2p has at least 40 % homology to SEQ ID NO:2 is found in the specification, for example, at page 11, lines 6-11.

The amendments to claim 37 concerning growth at a restrictive temperature are supported by, for example, the specification at page 14, line 29 through page 15, line 14. The control of growth by temperature can be used to select transformed cells without the need to incorporate an additional phenotype solely for the purpose of selection. Therefore, the simple fact that the cells grow at all at the restrictive temperature is selective for the desired mutant cells.

Support for new claims 43-45 is found in the originally filed claims 3, 25 and 36, respectively. Support for new claim 46 is found in originally filed claims12, 23 and 29.

§112 Rejections of the Claims

A. Written Description

Claims 1, 2, 5-13, 16-24, 27-35 and 37-42 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. In particular, the Examiner stated that the claims encompass any protein and/or nucleic acid sequence from any organism having structural homology with the nucleic acid sequence encoding Vff2p and/or the Vff2 protein.

Under the new "Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶1 Written Description Requirement," the written description requirement for a claimed genus may be satisfied by "disclosure of relevant, identifying characteristics, *i.e.*, structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus." Fed. Reg. 66:1099-1111, 1106, (January 1, 2001).

It should be noted that claims 1, 13, 24, 28, and 35 have been canceled, and that independent claims 3, 14, 25, 31, 33, 36, and 37 have been amended to expedite prosecution of the remaining claims in the present application. Insofar as the rejection is applied to the present claims, Applicants traverse the rejection.

The present claims recite polynucleotides, expression vectors, and host cells (and methods of using these), comprising a sequence encoding a functional Vff2p comprising SEQ ID NO:2, or a variant thereof. It is respectfully submitted that the present invention clearly satisfies the written description requirements as set forth in 35 U.S.C. 112, ¶1. It is respectfully submitted that those of skill in the art would readily recognize proteins and/or nucleic acid sequences which are encompassed by the present invention based on Applicants' specification. Applicants clearly set forth a structural and functional description of their invention. As discussed by the Examiner, those of skill in the art can readily identify nucleic acid sequence and/or proteins having structural homology to nucleic acid encoding Vff2p and/or the Vff2 protein, including those from other organisms. Based on Applicants' disclosure, those of skill in the art can further identify

whether the structural homologs are functionally homologous to the present invention, *i.e.*, involved in the secretory pathway and/or involved in the required cellular machinery for membrane fusion. Indeed, Applicants do not desire to claim every structural homolog of Vff2p, only those which are structurally and functionally homologous to the present invention, *i.e.*, Vff2p or variants thereof.

As discussed above, the term "variant" of Vff2p is defined in the specification as a polypeptide that is not completely identical to native Vff2p. Such a variant Vff2p can be obtained by altering the amino acid sequence be a structural homolog that has an insertion, deletion or substitution of one or more amino acids. *Id.* The amino acid sequence of the protein is modified, for example by substitution, to create a polypeptide having substantially the same or improved qualities as compared to the native polypeptide. *See* specification at page 9, line 7 to page 10, line 28. Thus, the polypeptides and polynucleotides of the present invention may have minor modifications (variations) from the native molecules but have substantially the same or improved qualities as compared to the native polypeptide. The application, therefore, discloses requisite relevant structural and functional characteristics of the molecules and cells, as the molecule structurally must be a Vff2p comprising SEQ ID NO:2 or a variant thereof, and functionally has substantially the same or improved qualities as compared to the native polypeptide.

Applicants therefore request that the rejection under 35 U.S.C. § 112, first paragraph (written description) be withdrawn.

B. Enablement

Claims 1-42 were rejected under 35 U.S.C. § 112, first paragraph, because the specification does not reasonably provide enablement for a polynucleotide with a sequence other than that of SEQ ID NO:1, a protein with a sequence other than that of SEQ ID NO:2, or a host cell other than *Saccharomyces cerevisiae*. Claims 1, 13, 24, 28, and 35 have been canceled, and independent claims 3, 14, 25, 31, 33, 36, and 37 have been amended to expedite prosecution of the presently remaining claims, and not for reasons of patentability, as discussed above. Insofar as the rejection is applied to the present claims, Applicants traverse the rejection.

To be enabling, the specification of a patent must teach those skilled in the art how to make and use the full scope of the claimed invention without undue experimentation. *Genentech Inc. v. Novo Nordisk A/S*, 108 F.3d 1361, 42 U.S.P.Q.2d (BNA) 1001, 1004 (Fed. Cir. 1997).

The scope of the claims must bear a reasonable correlation to the scope of enablement provided by the specification to persons of ordinary skill in the art. *Id.* Whether making or using the invention would have required undue experimentation, and thus whether the disclosure is enabling, is a matter of degree. *PPG Industries Inc. v. Guardian Industries Corp.*, 156 F.3d 1351, 37 U.S.P.Q.2d (BNA) 1618, 1623 (Fed. Cir. 1996). The fact that some experimentation is necessary does not preclude enablement; what is required is that the amount of experimentation must not be unduly extensive. *Id.*

Applicants assert that the present patent specification teaches one skilled in the art how to make and use the full scope of the claimed invention without undue experimentation. The scope of the claims must bear a reasonable correlation to the scope of enablement provided by the specification to persons of ordinary skill in the art. The pending claims recite a functional vesicular fusion factor 2 protein (Vff2p) wherein the Vff2p comprises SEQ ID NO:2 or a variant thereof. The present specification teaches the full sequence of SEQ ID NO:2, and specifically indicates how amino acids can be modified while still having substantially the same or improved qualities as compared to the native polypeptide. The specification extensively discusses various substitutions that can be made to a polynucleotide sequence without affecting the production of a functional protein (*see, e.g.,* page 8, line 17 through page 9, line 2*)*. Also, the specification extensively discusses modifications that can be made to the amino acid sequence that will still result in a functional protein (*see, e.g.,* page 9, line 7 through page 10, line 28). Therefore, with respect to the substitution of various nucleotides and/or amino acids, the art is actually quite predictable. Thus, the specification is enabling.

If the Examiner takes the view that the specification must not only indicate the starting material, but also teach how the modification is to occur (*e.g.,* substitution, deletion, or insertion), then Applicants still maintain that the specification is enabling. Some experimentation would be needed in order to test all the possible new proteins that could be made and be covered by Applicants' application. The amount of experimentation, however, would not be undue in view of teaching of the specification. The factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the Federal Circuit in *In re Wands*, 858 F.2d 731, 8 U.S.P.Q.2d (BNA) 1400, 1404 (Fed. Cir. 1988). They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the

invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.

As discussed above, the specification provides a significant amount of direction and guidance (**factor 2**). The specification extensively discusses various substitutions that can be made to a polynucleotide sequence without affecting the production of a functional protein (*see, e.g.,* page 8, line 17 through page 9, line 2). Also, the specification extensively discusses modifications that can be made to the amino acid sequence that will still result in a functional protein (*see, e.g.,* page 9, line 7 through page 10, line 28). An art worker in possession of Applicant's specification and having knowledge generally available to the art, would know how to make Vff2p. The art worker would also know how to transform cells from various species with the Vff2p polynucleotide or expression vector to make the claimed host cells.

The skill of those in the art (**factor 6**) is quite high in the field of molecular, as evidenced by the level of sophistication of the experiments set forth in the specification. Concerning the nature of the invention (**factor 4**), prior to the present invention,Vff2p was not known. The state of the prior art (**factor 5**) with respect to what was known about yeast proteins was well-developed, but there was nothing in the art relating to Vff2p. The breadth of the claims (**factor 8**) is commensurate with the disclosure. The claims recite a functional Vff2p, wherein the Vff2p comprises SEQ ID NO:2 or a variant thereof.

The Examiner states that the area of the invention in its full scope is unpredictable and would require undue experimentation by one of skill in the art to practice the invention. Regarding the quantity of experimentation necessary (**factor 1**) and the predictability or unpredictability of the art (**factor 7**), mathematically a large number of variant Vff2p molecules could be generated and screened. With respect to "undue experimentation," the fact that the outcome of a synthesis/screening program is unpredictable is precisely <u>why</u> a screening program is carried out. The Examiner simply cannot reasonably contend that a screening program to locate biomolecules with target biological properties would not be carried out by the art worker because the results cannot be fully predicted in advance. In fact, the Federal Circuit has explicitly recognized that a need to carry out extensive synthesis and screening programs to locate bioactive molecules does <u>not</u> constitute undue experimentation. *In re Wands*, at 1406-1407. In *Wands* the court held that a process of immunizing animals, fusing lymphocytes

from the immunized animals with myeloma cells to make hybridomas, cloning the hybridomas, and screening the antibodies produced by the hybridomas for the desired characteristics did not require undue experimentation. The Court stated:

> The nature of monoclonal antibody technology is that it involves screening hybridomas to determine which ones secrete antibody with desired characteristics. Practitioners of this art are prepared to screen negative hybridomas in order to find one that makes the desired antibody.

Likewise, practitioners having skill in the art related to the present application, given the teachings of the present specification, would be well-equipped to prepare and screen Vff2p polypeptides to locate additional Vff2p variants that retain the functional properties of native Vff2p (*i.e.*, increased cell growth) using the experimental protocol set forth in Example 3D. Thus, the fact that a claim may encompass a large number of variant Vff2p polypeptides is not dispositive of the enablement issue. This is particularly true in an art area in which the level of skill is very high. Practitioners in the art related to the present application would be well-equipped to probe for polypeptides from various yeasts to locate additional Vff2p-encoding polynucleotides. *See also, Hybritech Inc. v. Monoclonal Antibodies Inc.*, 231 U.S.P.Q. 81, 84 (Fed. Cir. 1986) (evidence that screening methods used to identify characteristics were available to art convincing of enablement). The preparation of variant Vff2p polypeptides is within the skill of the art. As Applicant has given the nucleotide and polypeptide sequence for Vff2p in the specification, one with skill in the art would easily be able to probe and/or search sequence databases to determine variants to the Vff2p indicated in SEQ ID NO:2.

Thus, considering the *Wands* factors, it would not require undue experimentation to obtain additional Vff2p molecules commensurate in scope with the pending claims. Applicants therefore assert that the specification fully enables one skilled in the art to make and use a functional Vff2p, wherein the Vff2p comprises SEQ ID NO:2 or a variant thereof.

In addition, concerning the existence of Vff2p molecules in other species of organisms, the Examiner states that the specification does not provide sufficient direction to support the claimed invention in its full scope with respect to other variants such as Vff2p homologs from other species besides *S. cerevisiae*. The specification, however, clearly indicates that a BLAST

analysis has been done in three very diverse organisms, *S. pombe*, *C. elegans* and *Arabidopsis*. Page 11, lines 1-11 of the specification states:

> Analyses of various databases have indicated that SEQ ID NO:1 and SEQ ID NO:2 are unique sequences in the yeast *S. cerevisiae*. By doing a BLAST analysis, homologous sequences have been identified in *S. pombe*, the nematode *Caenorhabditis elegans*, and the plant *Arabidopsis*. These homologous regions are simply open reading frames (ORFs) in the sequenced genome of these organisms. These ORFs have not been studied to date. Vff2p had 36% identity (*i.e.*, identical amino acids) and 56% homology (*i.e.*, the amino acids were of the same structure/function group, as described above) with *S. pombe*; had 25% identity and 46% homology with *Arabidopsis*; and 24% identity and 40% homology with *C. elegans*. It is quite surprising that there would be a high level of identity and homology in such diverse organisms.

The examiner states that "applicants admit that the percent identity for sequences in other species that may be homologous to Vff2p is low" (emphasis added). To the contrary, as quoted above, applicants state that there was a surprisingly ***high*** level of identity and homology in these organisms.

One with skill in the art would recognize that the levels of homology and identity found by the inventors was quite high. For example, in Tanaka *et al.*, Mol. Cell. Biol., 19:8660-8672 (Dec. 1999) (copy enclosed), the authors compared members of the Smt3p/SUMO-1 protein family to ubiquitin, and found that they were homologous to ubiquitin and played major regulatory roles of protein. *Id.* at page 8669. Even though these proteins were considered to be "homologous" the amino acid sequence comparisons revealed that *S. pombe* Pmt2p is 39% identical to *S. cerevisiae* Smt3P and human SMT3C (SUMO-1), and 11% identical to ubiquitin. *Id.* at page 8664. Thus, the 36% identity found between the Vff2p from *S. cerevisiae* and from *S. pombe* would be recognized as being high. Indeed, though not presently pursued in the present application, the 25% identity between the Vff2p from *S. cerevisiae* and *Arabidopsis*, and 24% identity between the Vff2p from *S. cerevisiae* and *C. elegans* would be recognized as quite high in organisms that are so diverse. Therefore, sufficient guidance is provided to support the pending claims (*Wands* factor 2).

The Examiner also raised the issue concerning isolating Vff2p from one species and expressing it in a host cell from another species. As discussed in detail previously, the Examiner admits that the relative skill level of those in the art of recombinant engineering and protein expression is high. It is well within the level of skill of the art worker, in conjunction with the disclosure of the present application, to clone Vff2p from one species and have it expressed in a host cell from another species. Many yeast and non-yeast sequences have been introduced into and expressed by yeast cells and other types of host cells.

The first paragraph of 35 U.S.C. §112 requires no more than a disclosure sufficient to enable one skilled in the art to carry out the invention commensurate with the scope of the claims, and this requirement has been met. It is respectfully submitted that the pending claims conform with 35 U.S.C. §112, first paragraph. Therefore, Applicant requests that the Examiner withdraw the 35 U.S.C. §112, first paragraph (enablement) rejection.

C. Indefiniteness

1. Claims 37-42

Claims 37-42 remain rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner states that claims are vague and indefinite in that they do not recite a positive process step that clearly relates back to the preamble. Independent claim 37 has been amended to clarify that the growing of the cells at a restrictive temperature is what allows for the selection of the mutant cells of the present invention. Applicant therefore requests that this rejection be withdrawn.

2. Claims 1-42

Claims 1-42 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 1-42 are indefinite for the recitation of the phrase "structural homolog(s)." This term has been deleted from the claims, thereby rendering this rejection moot. Applicant therefore requests that this rejection be withdrawn.

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612-373-6961) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

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CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, Washington, D.C. 20231, on this 16th day of April, 2001.

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